

REMARKS

Applicants have submitted herewith a Substitute Specification. The Substitute Specification does not contain new matter. The Substitute Specification adds paragraph identifiers and Sequence Listing identifiers and otherwise introduces amendments made during the international proceedings of the application. A marked-up copy of the original specification showing the matter being added to and deleted from the specification is also submitted herewith. The Claims have been amended to remove the multiple dependencies and to introduce amendments made during the international phase of the proceedings. Claims 1-31 have been canceled. Claims 32-62 have been added and so are pending in the application. This method of amendment was selected for its editorial convenience.

Conclusion

Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Mark J. Nuell, Ph.D. (Reg. No. 36,623) at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.14; particularly, extension of time fees.

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Respectfully submitted,

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Attachments: Substitute Specification (clean copy)
Substitute Specification (mark-up copy)
Substitute Abstract

SUBSTITUTE SPECIFICATION- MARKED-UP VERSION10/537264
JC17 Rec'd FEB/01 01 JUN 2005

"PHARMACEUTICAL COMPOSITIONS PREPARATION OF PEPTIDES,
SECRETED BY THE SNAKE VENOM GLANDS, PARTICULARLY OF BOTROPS
JARARACA, VASOPEPTIDASES INHIBITORS, EVASINS, THEIR ANALOGUES,
DERIVATIVES AND PRODUCTS ASSOCIATED, THEREOF. FOR DEVELOPMENT
5 OF APPLICATIONS AND USE IN CHRONIC-DEGENERATIVE DISEASES"

In the specification:

Please replace Paragraph [0001], with the following rewritten paragraph:

The present invention is characterized by the preparation
10 process of pharmaceutical compositions of peptides secreted by
the snake venom gland, particularly of the *Bothrops Jararaca*,
vasopeptidase inhibitors peptides, Evasins, SEQ No 1 to -
594595, their —analogues and derivatives and products
associated for development of application and/or associated
15 products for chronic-degenerative diseases.

[0002] It is further characterized by pharmaceutical
compositions and/or related products of vasopeptidase
inhibitors peptides, Evasins peptides and their structural
and/or conformational analogues and derivatives included in
20 cyclodextrins or their derivatives, or associated or included
in carriers and /or excipientes pharmacologically acceptable.
Another characteristic of this invention is the
microencapsulation of Evasins their analogues and derivatives
included or not in cyclodextrins, in controlled - release
25 systems such as liposomes and biodegradable polymers and
mixtures thereof.

[0003] The pharmaceutical compositions claimed in this patent
comprises the Evasins included in cyclodextrins or their
derivatives; or Evasins associated or included in carriers and
30 /or pharmaceutically acceptable excipients, alone or mixed or
associated at least with one pharmacologically active agent;
or Evasins included or not in cyclodextrins, microencapsulated

or not in controlled-release systems such us the liposomes and biodegradable polymers and/or mixtures thereof.

[0004] The present invention also comprises the identification of other biochemical mechanisms of action of Evasins having an 5 application in the study and treatment of chronic degenerative diseases.

Please replace Paragraph [0005], with the following rewritten paragraph:

The pharmaceutical compositions of the Evasins, and their 10 analogues and derivatives, except the Evasin 7a, SEQ No: 253, are characterized by the differential inhibitory activity for the neutral endopeptidase (K_i in the micro-molar range) and the Angiotensin I converting enzyme (K_i -in the nano-molar range). Another characteristic of these pharmaceutical 15 compositions there in is the increase of the biodisponibility, duration and / or efficacy of the Evasins effect when included in cyclodextrins, or their derivatives.

[0006] —The pharmaceutical compositions there in present an increase of the biodisponibility, duration and/ or efficacy of 20 the Evasins included in cyclodextrins, or their derivatives, when given by oral or venous route, as no limitante example.

[0007] In most of the countries worldwide, 15% to 25% of the adult population experience hypertension (MacMahon, S. et. Al. Blood pressure, stroke, and coronary heart disease, Lancet 25 335:765-774, 1990). The cardiovascular risk increases with the blood pressure level. The higher the blood pressure is, the higher is the risk of stroke and coronary events. Regarded as the main risk factor of coronary, cerebral and renal vascular diseases, hypertension is the main cause of death and 30 disability among adults.

[0008] Worldwide, heart failure is the main cause of hospitalization of patients 60 to 80 years old. The population ageing itself represents a factor of increased incidence,

while 1% of the individuals experience heart failure from 25 to 54 years old; among elder individuals. this incidence is much higher, reaching about 10% of those over 75 years (Kannel, W. B. et. al. Changing epidemiological features of 5 cardiac failure, Br. Hear J 1994; 72 (suppl): S3-S9).

| [0009] As per its clinical profile, heart failure is a limiting disease; when aggravated, the patients' quality of life decreases and, in more advanced cases, it has the features of a malignant disease having a mortality level above 10 60% in the first year, even nowadays (Oliveira, M.T. Características clínicas e prognóstico de pacientes com insuficiência cardíaca congestiva avançada, Faculdade de Medicina, USP 1999). It is currently estimated that more than 15 million people in the industrialized world are affected; 15 only in the USA, for example, it is estimated that the number of cases has increased 450% from 1973 to 1990 (Kannel, W.B. et. El. Changing epidemiological features of cardiac failure, Br. Hear J 1994; 72 (suppl 3): S3-S9).

| [0010] Hypertension is a complex, multifactorial and highly 20 prevalent disease, being responsible for several deleterious adverse effects and high morbidity/mortality (Kaplan, N. M. Blood pressure as a cardiovascular risk factor: prevention and treatment. JAMA. 275:1571-1576, 1996). A number of studies for evaluating the efficacy of its control in the general 25 population and special groups have been conducted, aiming a better understanding. The pressure control without a wide non-drug and/or pharmaceutical intervention with the associated risk factors (diabetes, obesity, smoking) may reduce or even eliminate the blood hypertension long-term treatment benefits 30 concerning mortality reduction, generally caused by coronary disease (Wilson, P. W. et. al. Hypertension, the risk factors and the risk of cardiovascular disease. Raven Press. 94-114).

[0011] Hypertension is the main contribution factor to cardiovascular arteriosclerosis (The fifth Report of the Joint National Committee on detection, evaluation, and treatment of High Blood Pressure. National Institute of Health (JNC).
5 Arch. Intern. Med. 153:154-181, 1994). As per the statistics, one of four Americans is or will be a hypertensive patient; 4.78 million are estimated to experience heart failure. Four hundred thousand new cases are diagnosed every year, leading to 800 thousand hospitalizations; US\$ 17.8 billion dollars are
10 spent with treatment.

[0012] In Brazil, data from SUS [Unified Health System] show that heart failure was the main cause of hospitalizations among the cardiac diseases in 1997; the government spent R\$ 150 millions in treatment, this amount being equivalent to
15 4.6% of the health expenses (Filho, Albarasi F. Insuficiência cardíaca no Brasil. Arq. Bras. Cardiol, 71:561-562, 1998).

[0013] The renin-angiotensin system (RAS) is responsible for regulating the blood pressure, cardiovascular homeostasis and hydroelectrolytic balance, both under physiological and
20 pathological conditions (Santos, R. A. S.; Campagnole-Santos, M. J.; Andrade, S. P. Angiotensin-(1-7): an update. Regulatory Peptides , 91:45-62, 2000). Angiotensin II (Ang II) is the main effector peptide of RAS, having vasopressor, adrenal steroids synthesis stimulating, proliferating (fibroblasts,
25 vascular smooth muscle) and hypertrophic (cardiac myocytes) actions. Its formation pathway involves the production of angiotensinogen by the liver and the production of renin in the justaglomerular system. These substances are released in the blood stream where the angiotensinogen is hydrolyzed by
30 renin, thus forming Ang I, that, when in the lungs, will undergo the action of the angiotensin converting enzyme (ACE) and generate Ang II. Ang II, in turn, will act on target organs remote from its production site (Santos, R. A. S.;

Campagnole-Santos, M. J.; Andrade, S. P. Angiotensin-(1-7): an update. *Regulatory Peptides*, 91:45-62, 2000).

| [0014] It was recently discovered that, in addition to the system that generates the circulating Ang II, different 5 tissues contain independent Ang II generating RAS, apparently by local action. The tissue RAS components are found on the walls of blood vessels, uterus, exocrine portion of pancreas, eyes, heart, adrenal cortex, testis, ovaries, pituitary gland anterior and intermediate lobes, pineal and brain. The 10 functions of these tissue SRAs are not very clear yet. (Ardaillou, R.; Michel, J. B. The relative roles of circulating and tissue renin-angiotensin systems. *Nephrol. Dial. Transplant.*, 14:283-286, 1999). The local actions of RAS may occur at the cell producing peptides (intracrine and 15 autocrine functions), on adjacent cells (paracrine function) or in a location away from the production site (endocrine function).

| [0015] Recent remarks indicate that important peripheral and central actions of RAS can be mediated by smaller 20 angiotensinergic peptides sequences, including Ang III [Ang-(2-8)], Ang IV [Ang-(3-8)] and Ang-(1-7). We can consider that both Ang I [Ang-(1-10)] and Ang II [Ang-(1-8)] can undergo a biotransformation process, thus giving raise to a "family" of biologically active angiotensin peptides. (Santos, R. A. S.; 25 Campagnole-Santos, M. J.; Andrade, S. P. Angiotensin-(1-7): an update. *Regulatory Peptides*, 91:45-62, 2000).

| [0016] Ang-(1-7), together with Ang II, are the main RAS 30 effectors. Two important characteristics segregate Ang-(1-7) from Ang II: the first has highly specific biologic actions and its formation pathway is independent from ACE (Santos, R. A. S.; Campagnole-Santos, M. J.; Andrade, S. P. Angiotensin-(1-7): an update. *Regulatory Peptides*, 91:45-62, 2000). Evasins would favor the formation of Ang-(1-7) by increasing

Ang I concentration and reduce its metabolism by inhibiting ACE.

| [0017] The primary purpose of the treatment of hypertension is not only to reduce the expenses, but also to prevent injuries 5 in target organs by changing the quality of life and the use of drugs when required (The Fifth Report of The Joint National Committee on detection, evaluation, and treatment of High Blood Pressure. National Institute of Health (VJNC). Arch. Intern. Med. 153:154-181, 1994).

10 | [0018] The drug treatment is indicated in cases of non-response to lifestyle changes after a term from three to six months and in case of injuries in target organs (left ventricular hypertrophy, myocardial ischemia, stroke or hypertensive retinopathy). All patients showing a systolic 15 blood pressure above 160 mmHg or diastolic blood pressure above 100 mmHg should be subject to pharmacological treatment, regardless of other factors whether present or not (Report the Canadian Hypertension Society. Consensus Conference. 3. Pharmacological treatment of essential 20 hypertension. Xan. Med. Assoc. J. 149 (3): 575-584, 1993).

| [0019] During the 70's and 80's, however, the antihypertensive drugs became an important tool in the treatment of high blood pressure (Ménard, J. Anthology of renin-angiotensin system: A one hundred reference approach to angiotensin II antagonist. 25 J. Hypertension 11 (suppl 3): S3-S11, 1993). During the last four decades, the pharmacological research produced new classes of drugs to treat hypertension: diuretics during the 60's, beta-blockers in the 70's, calcium channel blockers, antagonists of angiotensin II receptors and angiotensin 30 converting enzyme (ACE) inhibitors.

| [0020] Diuretics can be divided into three categories: thiazides loop and potassium sparing. Thiazides and the like include Chlorothiazide and Hydrochlorothiazide that reduce the

blood pressure in about 10 to 15% within the first days of treatment, this reduction being related to a decreased secondary extracellular volume and increased diuresis and natriuresis. Then, after six months, plasma volume and cardiac output return to normal values and the decreased blood pressure is related to a decreased peripheral vascular resistance (Frolich, E. Current approaches in the treatment of hypertension, 405-469). They are often used as a monotherapy, showing improved responses in black patients and, at low doses, in elder patients. The following side effects are seen: increased peripheral resistance to insulin, increased triglycerides, increased LDL, hypocalcemia, hyperuricemia. Furosemide, Bumetamide and Triamterene are among the loop diuretics showing higher potency than the thiazide diuretics. They primarily act on medullar and cortical portions of the Henle's loop. They show the same side effects of thiazide diuretics. The potassium sparing are drugs having a weak diuretic action, being rarely used alone. Among them, Amiloride, Triamterene and Spirolactone can be cited.

[0021] Beta-blockers, including Atenolol and Naolol, are classified as beta-1 and beta-2. The mechanism of antihypertensive action is not completely clear yet; however, it is basically supported by evidences that beta-blockers inhibit the presynaptic beta receptors, thus preventing the release of noradrenalin. The side effects include: change in the response to insulin, hypoglycemic coma extension, increased triglycerides and increased creatinine by reducing the renal flow.

[0022] The calcium channel blockers have been used for at least 25 years (Frolich, E. D. Current Approaches in the Treatment of Hypertension, 405-469, 1994). They can be divided into two large groups according to their pharmacological actions: those having increased action on the stimulus

conduction, such as Verapamil and Diltiazem and those having a predominant vasodilator action, such as the dihydropiridine derivatives (Nifedipine and others) (Frolich, E. D., Hypertension. Adult Clinical Cardiology Self Assessment Program (ACCSAP), 6: 3-19, 1995). Side effects include edema of lower limbs and tachycardia.

| [0023] The converting enzyme inhibitors primarily act by inhibiting the conversion of angiotensin I to angiotensin II. Thus, essentially vasoconstricting actions of angiotensin II are minimized. Preliminary studies show that teprotide (Evasin-9a), the first clinically used inhibitor has an antihypertensive activity when administered intravenously, however it is inactive when given orally, thus limiting its use. We currently know that ACE is an enzyme with multiple actions, that is, it acts on several substrates. In addition to act as a dipeptidase on angiotensin I and bradykinin, it is also able to disrupt peptide chains of the natriuretic peptide, indicating that the enzyme can act on several tissues. ACE has also an important role in the circulating and tissue Ang-(1-7) inactivation. The concentration of this circulating peptide is similar to the concentration of Ang II and is increased after the ACE inhibition. This increase can be a result of the increased precursor (Ang I) and decreased ACE-degradation (Santos, R. A. S.; Campagnole-Santos, M. J.; Andrade, S. P. Angiotensin-(1-7): an update. Regulatory Peptides , 91:45-62, 2000).

| [0024] The following patents were found in the state of the art that refer to the omapatrilat activity: US2002013307-A1, Kothari and Desai; US2002004500-A1 (WO200174348-A2, AU200187289-A), Bristol-Myers Squibb Co (BRIM) and Reeves et al.; US6166227-A (WO200003981-A2, AU9948528-A), Bristol-Myers Squibb Co(BRIM) and Godfrey et al. However, this vasopeptidase inhibitor compound and their analogs show relevant side

effects (angioedema and cough) as a result of the high inhibitory activity on the neutral endopeptidase (NEP).

[0025] The ACEI are excellent when administered as a monotherapy, since the ACE inhibitors lead to a relatively fast reduction of the blood pressure in 60 to 70% of the hypertensive patients (Ganong, W. *Neuropeptides in cardiovascular control.* J. Hypertens 2 (suppl 3): 15-22, 1984). They are generally well-tolerated, however their use may cause side effects and adverse reactions, some of them relatively severe and including angioneurotic edema, rashes and dry cough (8 to 10%), blood discrasias and sexual impotence.

[0026] The Angiotensin I converting enzyme inhibitors or the vasopeptidase inhibitors have been used or proposed to preventing or treating many diseases including tumors, acute myocardial infarction, stroke, left ventricular hypertrophy, diabetes angiopathy, peripheral ischemia, angina and progression of the heart failure after myocardial infarction and atherosclerosis, mellitus diabetes and angiogenesis.

20 (Yasumaru M, Tsuji S, Tsujii M, Irie T, Komori M, Kimura A, Nishida T, Kakiuchi Y, Kawai N, Murata H, Horimoto M, Sasaki Y, Hayashi N, Kawano S, Hori Inhibition of angiotensin II activity enhanced the antitumor effect of cyclooxygenase-2 inhibitors via insulin-like growth factor I receptor pathway;

25 Cancer Res. 2003 Oct 15;63(20):6726-34; Kinuya S, Yokoyama K, Kawashima A, Hiramatsu T, Konishi S, Shuke N, Watanabe N, Takayama T, Michigishi T, Tonami N. Pharmacologic intervention with angiotensin II and kininase inhibitor enhanced efficacy of radioimmunotherapy in human colon cancer

30 xenografts.J Nucl Med. 2000 Jul;41(7):1244-9.; Volpert OV, Ward WF, Lingen MW, Chesler L, Solt DB, Johnson MD, Molteni A, Polverini PJ, Bouck NP. Captopril inhibits angiogenesis and slows the growth of experimental tumors in rats.J Clin Invest.

1996 Aug 1;98(3):671-9.; Kowalski J, Herman ZS. Captopril augments antitumor activity of cyclophosphamide in mice. Pol J Pharmacol. 1996 May-Jun;48(3):281-5.; Kowalski J, Belowski D, Madej A, Herman ZS. Effects of thiorphan, bestatin and 5 captopril on the Lewis lung carcinoma metastases in mice. Pol J Pharmacol. 1995 Sep-Oct;47(5):423-7.; Kowalski J, Belowski D, Wielgus J, Gabryel B, Klin M, Herman ZS. Effect of captopril and thiorphan on the proliferation of human neoplastic cell lines and their influence on cytostatic activity of interferon 10 alpha or cytotoxic activity of doxorubicin. Arch Immunol Ther Exp (Warsz). 1995;43(1):47-50. However, it was not found in the state of the art any patent that claim the pharmaceutical composition of the Evasins included in cyclodextrins or not, formulated with carriers or excipients pharmacologically 15 acceptable with application in the study and treatment of degenerative chronic diseases.

Please replace Paragraph [0027], with the following rewritten paragraph:

[0027] The first attempts to develop Ang II antagonists are dated from the beginning of the 70's and were focused on the development of Ang II-analog peptides. The first, saralasin, 1-sarcosine, 8-isoleucine angiotensin II and then others. However, they were not clinically accepted, since they showed partial agonist activity. In 1982, the first two AT₁ receptors 20 and non-peptide antagonists were developed (S-8307 and S-8308) and, although having a highly specific and no agonist activity, showed a weak binding to Ang II receptors. After several changes in the molecular structure of these two parent compounds aiming to improve strength, keep selectivity and 25 reach pharmacokinetic properties, a new, potent and high-specificity oral product was developed, that is, Losartan. Since then, several other non-peptide antagonists were 30

developed, such as, Candesartan, Irbesartan, Valsartan, Telmisartan, Eprosartan, Tasosartan and Zolasartan.

[0027.1] The patent WO02/074782 A2, CA 2440749 A1 (2002) described the isolation and purification procedures of vasopeptidase peptide inhibitors, specific for the carboxylic site of the angiotensin enzyme, secreted by serpent venom glands (BPPs) or endogenously (EVASINS), presenting antihypertensive and vasodilator actions and the preparation of pharmaceutical compounds.

[0027.2] It was not find in the state-of-art the EVASINS formulation, SEQ No 1 to -594595, their analogues and derivatives and products associated, using cyclodextrins through the formation of the inclusion compounds, hereto claim. In addition it is not obvious obtain higher intravenous and oral biodisponibility of this kind of compounds, EVASINS formulation, SEQ No 1 to -594595, when included in cyclodextrins, claim of the present patent.

[0027.3] The pharmaceutical compositions and formulations of the present invention characterized by the use of a mixture of the pharmaceutically combined and acceptable excipients Evasins and analogs. Formulations can be prepared with an excipient or mixtures thereof. Examples of excipients include water, saline, phosphate-buffered solutions, Ringer's solution, dextrose solution, Hank's solution, biocompatible saline solutions whether containing polyethylene glycol or not. Other useful formulations include viscosity-increasing agents, such as sodium carboxymethylcellulose, sorbitol or dextran. The excipients can also contain lower amounts of additives, such as substances that increase the isotonicity and chemical stability or buffers. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer; examples of preservatives include thimerosal, meta- or ortho-cresol, formalin and benzyl alcohol. Standard formulations can be

liquid or solid. Thus, in a non-liquid formulation, the excipient may include dextrose, human serum albumin, preservatives, etc, to which water or sterile saline can be added before the administration.

5 | [0028] The present invention is further characterized by the preparation of controlled-release systems containing Evasins their analogs and derivatives. The satisfactory controlled-release systems include, but are not limited to cyclodextrins, biocompatible polymers, biodegradable polymers, other
10 polymeric matrixes, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres and transdermal administration systems. Other controlled-release compositions of the present invention include liquids that, after being administered to an animal,
15 form a solid or gel *in situ*.

| [0029] US4598070 (CA1215359, DK356684, EP135044, ES8506757, GR82322, JP60025967). Mashiro, Kawahara et. al. (1986) developed an invention referring to inclusion compounds between Tripudine (an antihypertensive) and cyclodextrins (α -cyclodextrin and β -cyclodextrin). Tripamide is slightly soluble in water and, thus, the use of cyclodextrins allowed more soluble compounds to be obtained. This invention also employed some characterization techniques, such as: differential scanning calorimetry (DSC) and X-rays
20 diffraction.
25

| [0030] US4666705. De Crosta, Mark. T. et. al. (1987) reported an invention based on the controlled release of hypertension drugs. Captopril, an ACE inhibitor (non-peptide, active site-directed ACE inhibitor) was used, since this inhibitor shows
30 fast absorption and a half-life of 2 hours. So as to retard the release, this invention was based on tablets containing captopril together with the polymer or copolymer. The polymer used was polyvinyl pyrrolidone (PVP) and dry granulation was

the technique. The result obtained was an increased indwelling time of the drug in the body (4 to 16 hours).

| [0031] US5519012. Fercej-Temeljoov, Darja et. al. (1996) developed an invention based on a new inclusion compound of a
5 antihypertensive, that is, 1,4-dihydropyridine, with methyl-
(β -cyclodextrin and other derivatives, such as (hydroxylated
 β -cyclodextrin).

| [0032] US5728402. Chen, Chih-Ming et. al. (1998) disclosed an invention based on the controlled release of drugs by means of
10 a pharmaceutical composition containing an inner phase comprising captopril and hydrogel, and another outer insoluble phase in the stomach. This invention intended to increase the drug absorption time that, when administered alone, is 1 hour.

| [0033] US5834432 (AU5990796, CA2221730, EP0828505, WO09639164,
15 JP115073625). Rodgers, Katlen Elizabeth et. al. (1998) used AT2 receptors agonists to accelerate wound healing.

| [0034] US6087386 (WO9749392A1). Chen, Tzyy-Show H. et. al. (2000) disclosed invention consisting in a pharmaceutical composition containing Enalapril (ACE inhibitor) and Losartan
20 (AII antagonist), comprising a layer of Losartan Potassium and another layer of Enalapril Maleate. This invention intended to improve the pharmacological action, reduce side effects and increase the absorption time.

| [0035] US6178349. Kieval, Roberts S. et. al. (2001) developed
25 a device based on the release of drugs by means of neural stimulation for the treatment of cardiovascular diseases. This device comprises an electrode connected to the nerve, an implantable pulse generator and a reservoir containing the drug to be administered. During use, the electrode and the
30 drug release stimulates the nerve that, in turn, affects the control on the cardiovascular system.

| [0036] A drug can be chemically modified so as to release its properties, such as biodistribution, pharmacokinetics and

solubility. Several methods have been used to increase the drugs' solubility and stability, including the use of organic solvents, emulsions, liposomes, pH adjustment, chemical changes and drugs complexation with an appropriate 5 encapsulating agent, such as cyclodextrins, liposomes and microencapsulation in biodegradable polymers.

| [0037] Cyclodextrins were first isolated in 1891 by Vilers, as the starch degradation products by the action of amylase of Bacillus macerans. In 1904, Schardinger characterized them as 10 cyclic oligosaccharides. In 1938, Frudenberg et al reported that cyclodextrins comprise glucose units joined by (α 1 \rightarrow 4) binding. In 1948, Freudenberg and colleagues observed that cyclodextrins are able to form inclusion compounds or complexes and, latter, such as French et al, prepared 15 synthesis processes of pure cyclodextrins. From 1954, Cramer et al conducted a systematic study on the formation of cyclodextrins complexes with other compounds. From 1955 to 1960, the first studies on the formation of cyclodextrins inclusion complexes with drugs were conducted. These studies 20 are intensively continued in Japan, Hungary, France, Italy and other countries.

| [0038] Cyclodextrins are obtained by the enzymatic degradation of starch. The methods comprise the following phases: enzyme production and purification, enzymatic transformation of 25 starch and recovery and separation of cyclodextrins. The enzyme involved is a cyclodextrin-glycosyltransferase (CGT). This enzyme is obtained from several microorganisms, but mainly Bacillus macerans, B. megatherium, B. stereothermophilus and Klebsiella pneumoniae. (Korolkovas, A. 30 Inclusão molecular e cyclodextrinas: propriedades e aplicações terapêuticas. ENLACE Farmalab, 2/91, Ano 5, Vol. II , p.6-15).

| [0039] Cyclodextrins are cyclic oligosaccharides that include six, seven or eight glucopyranose units. As a result of steric

interactions, the cyclodextrins, CDs, form a cyclic structure in the shape of a truncated cone having an internal apolar cavity. It includes chemically stable compounds that can be region-selectively modified. The cyclodextrins (hosts) form 5 complexes with several hydrophobic molecules (guest matter), completely or partially including them in the cavity. The CDs have been used for the solubilization and encapsulation of drugs, perfumes and aromatic agents as described by Szejtli, J., Chemical Reviews, (1998), 98, 1743-1753. Szejtli, J., J. 10 Mater. Chem., (1997), 7, 575-587. According to detailed toxicity, mutagenicity, teratogenicity and carcinogenicity studies on cyclodextrins, described in [Rajewski, R.A., Stella, V., J. Pharmaceutical Sciences, (1996), 85, 1142-1169], they have a low toxicity, especially the hydroxypropyl- 15 (-cyclodextrins, as reported in Szejtli, J. Cyclodextrins: Properties and applications. Drug Investig., 2 (suppl. 4): 11-21, 1990. Except for high concentrations of some derivatives that damage erythrocytes, these products generally impose no health risks. The use of cyclodextrins as additives in 20 foodstuff has been authorized in countries such as Japan and Hungary and for more specific applications in France and Denmark. All these characteristics mean an increasing motivation for the discovery of new applications.

| [0040] In addition to cyclodextrins, biodegradable polymers 25 are also used, which decrease the absorption speed of the drugs in the body by means of the controlled-release devices. In these systems, the drugs are incorporated in a polymeric matrix based in the encapsulation of the drugs in microspheres or nanospheres that release the drug inside the body in small 30 and controllable daily doses for days, months or even years.

| [0041] Several polymers have already been tested in controlled-release systems. Several of them as a function of their physical properties, such as: poly(urethanes) due to its

elasticity, poly(siloxanes) or silicone for being a good isolating agent, poly(methyl-methacrylate) as a result of its physical strength, poly(vinyl alcohol) as a result of its hydrophobicity and resistance, poly(ethylene) by virtue of its 5 hardness and impermeability (Gilding, D. K. Biodegradable polymers. *Biocompat. Clin. Implat. Mater.* 2: 209-232, 1981).

| [0042] Nevertheless, for use in humans, the material should be chemically inert and free of impurities. Some of the materials 10 used on release systems include: poly(2-hydroxyethylmethacrylate), polyacrylamide, lactic acid-based (PLA) and glycolic acid-based (PGA) polymers and the corresponding copolymers, (PLGA) and the poly (anhydrides), such as the sebasic acid- based (PSA) polymers and copolymers with hydrophobic polymers.

15 | [0043] The prior art relates several patents for the preparation of liposomes [US Patent 4,552,803, Lenk; US Patent 4,310,506, Baldeschwieler; US Patent 4,235,871, Papahadjopoulos; US Patent 4,224,179, Schneider; US Patent 4,078,052, Papahadjopoulos; US Patent 4,394,372, Alfaiate; US 20 Patent 4,308,166, Marchetti; US Patent 4,485,054, Mezei; and US Patent 4,508,703, Redziniak; Woodle and Papahadjopoulos, Methods Enzymol. 171:193-215 (1989)].

| [0044] Unilamellares liposomes have a single membrane that 25 contains an aqueous volume [Huang, Biochemistry 8:334-352 (1969)], while the multilamellar liposomes have several concentrical membranes [Bangham et Col., J. Mol. Biol. 13:238-252 (1965)]. Liposomes-based carriers were proposed for a variety of pharmacologically active substances, including antibiotics, hormones and antitumor agents [Medical 30 applications of liposomes (D.D. Lasic, D. Papahadjopoulos Ed.), Elsevier Science B.V., Holland, 1998].

| [0045] Other preparation processes of liposomes have been found in the prior art, [for a review, see, for example,

Cullis et al., in: *Liposomes, From Biophysics to Therapeutics* (M. Ostro, ed.), Marcel Dekker (New York), 1987, pp. 39-72; Woodle and Papahadjopoulos, *Methods Enzymol.* 171:193-215 (1989); *Liposome technology* (G. Gregoriadis ed.), CRC Press, 5 Boca Raton, FL, 1993].

| [0046] The Bangham's procedure [J. Mol. Biol. 13:238-252 (1965)] produces "ordinary multilamellar liposomes" (MLVs). The "ordinary" MLVs can have an unequal solute distribution among the aqueous compartments and thus show an osmotic 10 pressure differential among the compartments. Lenk et Col. (US Patent 4,522,803; US 5,030,453 and US 5,169,637), Fountain et al. (US Patent 4,588,578), Cullis et al. (US Patent 4,975,282) and Gregoriadis et al. (Patent WO99/65465) discovered methods 15 for the preparation of multilamellar liposomes having a substantially equal solute distribution among the compartments. An equal solute distribution among the different compartments means a greater drug encapsulation efficacy, as well as a lower osmotic pressure differential, then rendering these MLVs more stable than the ordinary MLVs.

20 | [0047] Unilamellar liposomes can be produced by sonication of MLVs [see Paphadjopoulos et al. (1968)] or by extrusion through polycarbonate membranes [Cullis et Col. (US Patent 5,008,050) and Loughrey et Col. (US Patent 5,059,421)]. Satisfactory lipids include, for example, phosphatidylcholine, 25 phosphatidylserine, phosphatidylglycerol, cardiolipin, cholesterol, phosphatidic acid, sphingolipides, glycolipids, fatty acids, sterols, phosphatidylethanolamine, polymerizable phospholipids in their declared polymerized or non-polymerized form, and mixtures thereof.

30 | [0048] The liposomes compositions of the present invention are characterized by a modification of vesicles, thus providing specificity to organs or cells. Liposomes direction was classified based on anatomical factors and mechanisms

involved. The anatomical classification is based on the selectivity level, for example, organ-specific, cell-specific or organelle-specific. From the mechanisms point of view, directing can be ranked as passive or active.

5 | [0049] Passive directing uses the natural trend of conventional liposomes to be captured by cells of the reticuloendothelial system in organs containing sinusoidal capillaries. The liposomes of the present invention are sterically established by the (LEE) method (also known as
10 "PEG-liposomes") as an improved drug carrier, due to its reduced elimination speed into blood stream [Lasic and Martin, Stealth Liposomes, CRC Press, Inc., Boca Raton, Fla. (1995)]. LEE are liposomes, the surface of which is covered by a polymer, this polymer being preferably polyethylene glycol
15 (PEG), covalently conjugated to one of the phospholipids and generates a hydrophilous cloud out of the vesicle double layer. This steric barrier retards the liposomes recognition by opsonins and allows LEE to remain in the blood stream for a longer time than the conventional liposomes [Lasic and Martin,
20 Stealth Liposomes, CRC Press, Inc., Boca Raton, Fla. (1995); Woodle et Col., Biochim. Biophys. Acta 1105:193-200 (1992); Litzinger et Col., Biochim. Biophys. Acta 1190:99-107 (1994); Bedu Addo, et Col., Pharm. Res. 13:718-724 (1996)] and increase the pharmacological efficacy of encapsulated agents,
25 as showed for some chemotherapeutic drugs [Lasic and Martin, Cautela Liposomes, CRC Press, Inc., Boca Raton, Fla. (1995)] and bioactive peptides [Allen T.M. In: Liposomes, New Systems, New Trends in their Applications (F. Puisieux, P. Couvreur, J. Delattre, J.-P. Devissaguet Ed.), Editions de la Santé,
30 França, 1995, pp. 125].

| [0050] Studies in this field demonstrated that different factors affect the circulation half-life of LEE and, ideally, the vesicles diameter should be lower than 200 nm, the PEG

having a molecular weight of about 2,000 Da at a ratio of 5% [Lasic and Martin, Cautela Liposomes, CRC Press, Inc., Boca Raton, Fla. (1995); Woodle et Col., Biochim. Biophys. Acta 1105:193-200 (1992); Litzinger et Col., Biochim. Biophys. Acta 5 1190:99-107 (1994); Bedu Addo et Col., Pharm. Res. 13:718-724 (1996)].

| [0051] Active directing involves the change of liposomes by means of its association with a binder, such as a monoclonal antibody, sugar, glycolipid, protein, polymer or by changing 10 the composition or size of liposomes to direct them towards organs and cells different from sites where the conventional liposomes accumulate. See, for example, Remington's Pharmaceutical Sciences, Gannaro, A. R., ed., Mack Publishing, 18th edition, pp. 1691-1693.

15 | [0052] An example found in the state of the liposomes application technical to prolong the effect of the peptide is the preparation of the liposomes containing Ang-(1-7) (Lang) unilaterally microinjected (in the rostro-ventrolateral medulla (RVLM). The blood pressure was measured by telemetry 20 for 10 seconds, every 10 minutes, starting 4 days before and finishing 12 days later in undisturbed rats with free movement. The Lang microinjection produced a significant pressor effect during the morning period and maintained for 5 days. The highest MBP was measured on day 3 (114 ± 4 mmHg) 25 that was significantly different from that measured on day 0 (100 ± 3 mmHg). As expected, the Lvaz did not change significantly in MBP (94 ± 5 mmHg on day 3 vs 90 ± 5 mmHg on day 0). Additionally, morning MBP was significantly higher in the Lang group than in the Lvaz group on days 1, 2 and 3. 30 Contrary to the morning MBP, the night MBP was not significantly affected by the Lang microinjection. Previous studies (Fontes MA, Pingue MC, Naves V, Campagnole-Santos MJ,

Lopes OU, Khosla MC, Santos RAS Cardiovascular effects produced by microinjection of angiotensins and angiotensin antagonists into the ventrolateral medulla of freely moving rats. *Brain Res.* 1997 Mar 7;750(1-2):305-10) established that
 5 the free Ang-(1-7) microinjection (not encapsulated) on BRVL at a similar dose (25 to 50 ng), leads to an increase of 15 mmHg for approximately 10 min. The short length of this effect was due to the peptide high metabolism *in vivo* [Silva-Barcellos et Col., *Hypertension*, 38(6): 1266-71 (2001)].

10 | Please replace Paragraph [0053], with the following rewritten paragraph:

The present invention is characterized by the use of at least twenty-one bradykinin potentiating peptides found in the venom and tissues of Bothrops jararaca (generically called BPPs or
 15 bradykinin potentiating peptides) had their amino acids sequences determined by mass spectrometry or deduced in cDNA of parent compounds of these molecules expressed in non-venom gland tissues of this serpent (called **Evasins** or **Endogenous Vasopeptidase Inhibitors**), SEQ No 1-594595.

20	[0054]	Nomenclature	Sequence
	ID 1	EVASIN-5a	<EKWAP
	ID 2	EVASIN-5b	<EWPRP
	ID 3	EVASIN-5c	<EKFAP
	ID 4	EVASIN-6a	<ESWPGP
25	ID 5	EVASIN-7a	<EDGPIPP
	ID 6	EVASIN-9a	<EWPRPQIPP
	ID 7	EVASIN-9b	<ESWPGNIPP
	ID 8	EVASIN-10a	<ESWPGPNIIPP
	ID 9	EVASIN-10b	<ENWPRPQIPP
30	ID 10	EVASIN-10c	<ENWPHPQIPP
	ID 11	EVASIN-10d	<ESWPEPNIPP
	ID 12	EVASIN-11a	<EWPRPTPQIPP
	ID 13	EVASIN-11b	<EGRAPGPPIPP

	ID 14	EVASIN-11c	<EGRAPHPPIPP
	ID 15	EVASIN-11d	<EGRPPGPPIPP
	ID 16	EVASIN-11e	EARPPHPIPP
	ID 17	EVASIN-12a	<EGWAWPRPQIPP
5	ID 18	EVASIN-12b	<EWGRPPGPPIPP
	ID 19	EVASIN-13a	<EGGWPRPGPEIPP
	ID 20	EVASIN-13b	<EGGLPRPGPEIPP
	ID 21	EVASIN-13c	<EGGWPRPGPQIPP-
		<u>Nomenclature</u>	<u>Sequence</u>
10	ID 1	EVASIN-5a	<EKWAP
	ID 2	EVASIN-5b	<EWPRP
	ID 3	EVASIN-5c	<EKFAP
	ID 4	EVASIN-6a	<ESWPGP
	ID 5	EVASIN-7a	<EDGPIPP
15	ID 6	EVASIN-9a	<EWPRPQIPP
	ID 7	EVASIN-9b	<ESWPGNIPP
	ID 8	EVASIN-10a	<ESWPGPNIIPP
	ID 9	EVASIN-10b	<ENWPRPQIPP
	ID 10	EVASIN-10c	<ENWPHPQIPP
20	ID 11	EVASIN-10d	<ESWPEPNIPP
	ID 12	EVASIN-11a	<EWPRPTPQIPP
	ID 13	EVASIN-11b	<EGRAPGPPIPP
	ID 14	EVASIN-11c	<EGRAPHPPIPP
	ID 15	EVASIN-11d	<EGRPPGPPIPP
25	ID 16	EVASIN-11e	EARPPHPIPP
	ID 17	EVASIN-12a	<EGWAWPRPQIPP
	ID 18	EVASIN-12b	<EWGRPPGPPIPP
	ID 19	EVASIN-13a	<EGGWPRPGPEIPP
	ID 20	EVASIN-13b	<EGGLPRPG
30	ID 21	EVASIN-13c	<EGGWPRPGPQIPP PEIPP

[0055] Most of these peptides have the structural motif C-terminal PX¹X²PP, wherein X¹ can be any amino acid and X² is typically a residue of isoleucine (I) and the N-terminal amino

acid is blocked, usually by the presence of a residue of pyroglutamic acid (<E>). The corresponding synthetic peptides were tested as C and N site inhibitors of recombinant ACE and as potentiators both of the bradykinin contractile activity in
 5 guinea pig isolated ileum and the hypotensive activity of bradykinin in rats. The most selective and effective as potentiators of the bradykinin contractile action in guinea pig isolated ileum and the hypotensive action on blood pressure in rats were those having masses between 500 and 1700
 10 Daltons, containing 5 to 13 amino acids residues. The active molecules were chemically modified, thus giving raise to other peptides having qualitatively similar characteristics.

Please replace Paragraph [0056], with the following rewritten paragraph:

15 The Evasins, oligopeptides of 5 to 13 amino acids, formulated therein, are those described below:

a) oligopeptides of 5-13 amino acids

EVASIN-5, SEQ ID No.1 to SEQ ID No. 103,

EVASIN-6, SEQ ID No. 104 to SEQ ID No. 243,

20 EVASIN-7, SEQ ID No. 244 to SEQ ID No.325,

EVASIN-9, SEQ ID No.326 to SEQ ID No.422 and SEQ ID No.595,

EVASIN-10, SEQ ID No.423 to SEQ ID No. 516,

EVASIN-11, SEQ ID No. 517 to SEQ ID No. 556,

EVASIN-12, SEQ ID No.557 to SEQ ID No.589,

25 EVASIN-13, SEQ ID No.590 to SEQ ID No.594594;

~~Or could be indentified from a sequence numbers as:~~

10 EVASIN-5a Xaa Lys Trp Ala Pro

22 EVASIN-5b Xaa Trp Pro Arg Pro

16 EVASIN-5c Xaa Lys Phe Ala Pro

30 167 EVASIN-6a Xaa Ser Trp Pro Gly Pro

253 EVASIN-7a Xaa Asp Gly Pro Ile Pro Pro

326 EVASIN-9a Xaa Trp Pro Arg Pro Gln Ile Pro

Pro

	EVASIN 9b	Xaa Ser Trp Pro Gly Asn Ile Pro
Pro		
423	EVASIN 10a	Xaa Ser Trp Pro Gly Pro Asn Ile
Pro Pro		
5	464 EVASIN 10b	Xaa Asn Trp Pro Arg Pro Gln Ile
Pro Pro		
472 EVASIN 10c	Xaa Asn Trp Pro His Pro Gln Ile	
Pro Pro		
10	447 EVASIN 10d	Xaa Ser Trp Pro Glu Pro Asn Ile
Pro Pro		
552 EVASIN 11a	Xaa Gly Arg Ala Pro Gly Pro Pro	
Ile Pro Pro		
553 EVASIN 11b	Xaa Gly Arg Ala Pro His Pro Pro	
Ile Pro Pro		
15	554 EVASIN 11c	Xaa Gly Arg Pro Pro Gly Pro Pro
Ile Pro Pro		
555 EVASIN 11d	Xaa Ala Arg Pro Pro His Pro Pro	
Ile Pro Pro		
558 EVASIN 12a	Xaa Gly Trp Ala Trp Pro Arg Pro	
Gln Ile Pro Pro		
20	557 EVASIN 12b	Xaa Trp Gly Arg Pro Pro Gly Pro
Pro Ile Pro Pro		
590 EVASIN 13a	Xaa Gly Gly Trp Pro Arg Pro Gly	
Pro Glu Ile Pro Pro		
25	592 EVASIN 13b	Xaa Gly Gly Leu Pro Arg Pro Gly
Pro Glu Ile Pro Pro		
590 EVASIN 13c	Xaa Gly Gly Trp Pro Arg Pro Gly Pro	
Gln Ile Pro Pro		
30	[0057] Another feature of the present invention is the possibility of a modification of all EVASINS molecules aiming to improve their pharmacokinetic and action specificity properties on different target molecules involved in cardiovascular pathologies, both as vasopeptidases inhibitors	

and as an action on endothelial cells and vessels smooth muscle by means of chemicals changes consisting in:

- 1) **Localized conformational changes** by substituting the L-amino acids with D-amino acids, leading to the introduction of a reverse β -“turn” structure (“hairpin”), or by introducing α -substituted amino acids, thus rendering the polypeptide chain axis intro a α -helix structure or extended conformation or β -“turn”, for example, the introduction of the α -aminoisobutyric. It is also provided a N-methyl substitution of a α -amino acid, thus restricting the action of the amide bond, eliminating the formation of a hydrogen bond, affecting the main polypeptide chain angle of torsion and allowing the formation of a cis peptide bond. Another modification is the peptide amide bond substitution with a non-amide covalent bond so as to protect this bond against the action of proteases.
- 2) **Global Conformational Changes** by cyclization, thus stabilizing secondary structures. The two amino acids chosen for the substitution with cysteine or other organic compounds containing, for example, a thiol group each with a β,β -dimethylpenicillinalanine analog, can be any of the amino acids residues of the EVASINs sequences or their analogs, being separated from each other by at least two polypeptide chain amino acids residues. The formation of a S-S bond is then favored between the two thiol residues, for example, thus forming a cyclic peptide. Cyclization can be also obtained by forming a lactam bond or peptide bond between the polypeptide free carboxylic and amino groups or any other chemical procedure that favors the peptide cyclization.

3) **Changes in amino acids side chains (χ -“constraints”).** The determination of the α -amino acids side chains angles of torsion may allow the topological changes that better adjust the polypeptide to its binding site, for example, the substitution of tyrosine with β -methyl-2',6'-dimethyltyrosine (TMT) that can define a preferential conformation to the interaction site. The proposed changes were referred and exemplified in the review of Victor J. Hruby published in Nature, 1, 847-858, 2002.

10 | [0058] The present invention is characterized by obtaining oligopeptides release systems, EVASINs, using cyclodextrins and their derivatives that reduce the degradation by the gastrointestinal tract (GIT), leading to increased bioavailability of the peptide in the biologic system particularly for orais formulations. Additionally, there are others applications forms like; intravenous, intramuscularly, topical, pulmonary inhalation, intranasal, intramounth or as a controlled liberation diapositive using biodegradable polymers as PLA and PLGA or mixture of these examples no limitants.

15 | [0059] The present invention is further characterized by controlled-release systems of oligopeptides, Evasins, using the liposomes that increase the peptide bioavailability. Liposomes are lipid vesicles that include internal aqueous compartments in such molecules, for example, drugs can be 20 encapsulated aiming to reach a slow drug release after the administration of the liposome to an individual.

| [0060] No application using oligopeptides, Evasins, or their structural and/or conformational analogs included in cyclodextrins or their derivatives, microencapsulated into 25 biodegradable polymers such as PLA ou PLGA ou mixed and the liposomes, was previously described. The present invention is characterized by the use of three different technologies, that

is, molecular encapsulation of oligopeptides, Evasins, and their analogs into cyclodextrins and the microencapsulation into biodegradable polymers or liposomes and/or mixtures thereof. allowing the increase of the biodisponibility of the
5 Evasins in the oral compositions and formulations when compared to not formulated.

Please replace Paragraph [0060], with the following rewritten paragraph:

None pharmaceutical composition and/or formulation of the
10 Evasins and their structural analogues and/or conformational characterized by the utilization of Evasins 7a, SEQ ID No.253,
EVASIN-10c, SEQ ID No.472, 11e, SEQ ID No. 555, 12b, SEQ ID
No. 557 the Evasins 7a, 10c, 11e, 12b and their
15 respectivesrespective analogues and derivatives as molecular models for development of drugs and /or pharmaceutical composition or formulations based on peptide compound and/or no-peptide was previously described.

[0061] Another feature of this invention is the use of the pharmaceutical compositions and/or formulations of the Evasins and their analogues and derivatives characterized by inclusion and/or association compounds among the Evasins and their analogues and derivatives cyclodextrins, their derivatives, microencapsulate or not in controlled-release systems such as liposomes and the biodegradable polymers PLA, PLGA and/or mixtures, relies on the use for the study and treatment of hypertension, other cardiovascular diseases and their complications (no limitant examples: acute myocardial infarction, stroke, left heart failure, diabetes angipathy, peripheral ischemia, angina and progression of the heart
25 failure after a myocardial infarction and atherosclerosis) tumors, diabetes melitus, sperm motility and spermatogenesis blocking, nephropathies, sexual impotence, gastrointestinal and gynecologic disorders, angiogenesis, hair loss, blood

diseases and angioplasty (post-angioplasty restenosis, endovascular protese) in hot-blood animals.

[0062] The pharmaceutical compositions claimed therein comprises the Evasins included in cyclodextrins or their derivatives, or Evasins their analogues and derivatives associated or included in carriers and /or excipients pharmaceutically acceptable, alone or mixed or associated at least with one pharmacologically active agent, or Evasins their analogues and derivatives included or not in cyclodextrins, microencapsulated or not in controlled-release systems such us the liposomes and PLA, PLGA biodegradable polymers and/or mixtures.

Please replace Paragraph [0063], with the following rewritten paragraph:

15 The present invention also comprises the identification of other biochemical mechanisms of action of Evasins, SEQ ID No. 1 to SEQ ID No. 595, having an application in the study and treatment of chronic degenerative diseases.

Please replace Paragraph [0064], with the following rewritten paragraph:

20 The pharmaceutical compositions of the Evasins, SEQ ID No. 1 to SEQ ID No. 595 and their analogues and derivatives, except the Evasin 7a, SEQ ID No. 253, there in are characterized for presenting diferential inhibitory activity for the neutral 25 endopeptidase (Ki in the micro-molar range) and the Angiotensin I converting enzyme (Ki -in the nano-molar range). Another characteristic of these pharmaceutical compositions and formulations is the increase of the biodisponibility, duration and / or efficacy of the cited 30 peptide effect when included in cyclodextrins, for example in a oral formulation.

[0065] —The present invention may be better understood based on the following no limitant examples.

**Example 1: SYNTHESIS, PURIFICATION AND CHARACTERIZATION
AND ENZYMATIC ASSAY TO DETERMINE THE SELECTIVE INHIBITION OF
CATALITICS SITE OF THE ANGIOTENSIN I CONVERTING ENZIME BY
EVASIN**

- 5 | [0066] This example describe the synthese, purification, characterization and site C or N-terminal selective inhibition of the ECA by Evasins. The oligopeptides were synthesized according to the state of art of the methodology. The strategy Boc is based on the data described in Barany, G.
- 10 | & Merrifield, R.B. (Gross, E. & Meinhofer, J., Eds.) (1980), The Peptides: Analysis, Synthesis and Biology, vol. II, 1, Academic Press, New York. 2-Stewart, J.M. & Young, J.D. (1984), Solid Phase Peptide Synthesis, Pierce Chemical Company, Rockford.
- 15 | [0067] The oligopeptides were modified according to a methodology of the state of the art. The proposed changes were referred and exemplified in the review of Victor J. Hruby published in the journal Nature, 1, 847-858, 2002.
- 20 | [0068] The synthetic Evasins were purified in a HPLC system and the eluded material was analysed by mass spectrometry.
- | [0069] In the purification the solvents utilized were all of the HPLC grade, degree) and the water utilized was obtained by distillation and filtration in the Milli-Q system, equipped with to cartridge to retain salt and the organic compound.
- 25 | [0070] The fractions from purification were submitted to mass spectrometry analysis to conforming the Evasins molar mass after purification.
- | [0071] The enzyme assays for the recombinant ACE inhibition were conducted using the substrate Mca-Ala-Ser-Asp-Lys-DpaOH,
- 30 | at 25°C, in a 50 mM Hepes buffer (pH 6.8), 200 mM NaCl and 10 µM ZnCl₂. The reactions were continuously monitored by determining the fluorescence increase at $\lambda_{em} = 390$ nm ($\lambda_{ex} =$

340 nm), provided by the substrate cleavage ($S = Km$, 40 μM) by ACE in a fluorometer. The Evasins were pre-incubated with the enzyme before the substrate addition.

5 | Please replace Paragraph [0072], with the following rewritten paragraph:

The results of K_i values for the ACE inhibition by Evasins were in the order of nM. Most of the Evasins were selective inhibitors for the C-terminal site, Evasin-10c, SEQ ID No. 472, for example, showed a K_i value of 0.5 nM, that is, 400 times more selective for this dominium. While other Evasins showed selective inhibition for the N-terminal site, for example, the K_{is} values obtained using Evasin-12b, SEQ ID No. 557, were 5 nM and 150 nM for the N- and C-terminal sites, respectively.

10 | 15 | Please replace Paragraph [0073], with the following rewritten paragraph:

Table 1: Assay of the inhibition of the activity of the C and N-terminal catalytic site of the Angiotensin I converting enzyme by the Evasins.

PEPTIDE	ECA (nM) - K_i	
	C-terminal	N-terminal
EVASIN-5a, <u>SEQ ID No. 10</u> ^a	1280	399
EVASIN-7a, <u>SEQ ID No. 253</u> ^a	40000	70000
EVASIN-9a, <u>SEQ ID No. 326</u> ^a	1	100
EVASIN-10 ^a , <u>SEQ ID No. 423</u> ^a	33	130
EVASIN-10c, <u>SEQ ID No. 472</u>	0,5	200
EVASIN-11b, <u>SEQ ID No.</u>	57	3000

<u>552</u>		
EVASIN-11c, SEQ ID No.	40	2000
<u>553</u>		
EVASIN-11e, SEQ ID No.	300	100
<u>555</u>		
EVASIN-12a, SEQ ID No.	25	25
<u>558^a</u>		
EVASIN-12b, SEQ ID No.	150	5
<u>557</u>		
EVASIN-13aa, SEQ ID No.	50	50
<u>590</u>		

[0074] Considering the state of the art, it was demonstrated the mice with Angiotensin I converting enzyme genetic deletion are infertile (. (Esther CR Jr, Howard TE, Marino EM, Goddard JM, Capecchi MR, Bernstein KE. Mice lacking angiotensin-converting enzyme have low blood pressure, renal pathology, and reduced male fertility. *Lab Invest.* 1996 May;74(5):953-65) and the testicular ACE corresponds to the ACE somatic N-terminal site (Ramaraj P, Kessler SP, Colmenares C, Sen GC Selective restoration of male fertility in mice lacking angiotensin-converting enzymes by sperm-specific expression of the testicular isozyme. *J Clin Invest.* 1998 Jul 15;102(2):371-8), Pharmaceutical compositions of the Evasin, their analogues and derivatives have the potential to be utilized as male contraceptive. Thus, the present invention also is characterized by the mixture of cyclodextrins organic-aqueous or solid solutions or cyclodextrins derivatives from the alquil, hydroxialquil, hydroxipropil e acyl group with cross bond or cyclodextrins polymers with organic-aqueous or solid solutions of the Evasins and/ or of its analogues to be used as a male contraceptive. On the other hand, the Evasins with the selectivity to the C-terminal site may present distinct

characteristic different from the ones presented in the inhibitors not selective or selective to the N site.

Example 2: ENZYME ASSAYS FOR DETERMINING THE EVASINS-INDUCED NEP INHIBITION

5 | [0075] The fluorometric assays for the recombinant NEP inhibition were conducted using the substrate Abz-RL-EDDnp in a 50 mM Tris-HCl buffer, pH 7.5, at 37°C. The reactions were continuously monitored by determining the fluorescence increase at $\lambda_{em} = 418$ nm ($\lambda_{ex} = 318$ nm), provided by the
10 | substrate cleavage ($K_m = 8.4 \mu M$) by NEP in a fluorometer. The Evasins were pre-incubated with the enzyme before the substrate addition.

15 | [0076] The Evasins are not very potent NEP inhibitors, the results of K_i values for the Evasins-induced NEP inhibition were in the order of μM . One of the best inhibitors was Evasin-9a, that showed a K_i value of 86 μM .

| Please replace Paragraph [0077], with the following rewritten paragraph:

Table 2: Assay of the neutral endopeptidase activity
20 | inhibition by the Evasin action

PEPTIDE	NEP (μM) - K_i
EVASIN-5a, SEQ ID No. 10 ^a	550
EVASIN-7a, SEQ ID No. 253 ^a	492
EVASIN-9a, SEQ ID No. 326 ^a	86
EVASIN-10c, SEQ ID No. 472	253
EVASIN-11b, SEQ ID No. 552	476
EVASIN-11c, SEQ ID No. 553	589
EVASIN-12a, SEQ ID No. 558 ^a	106
EVASIN-12b, SEQ ID No. 557	150

EVASIN-13a, SEQ ID No. 590

473

[0078] The inhibition constants (K_i) values were determined by means of the ratio of the apparent inhibition constant ($K_{i(app)}$) and the substrate K_m (Salvesen and Nagase, 1990). In: Proteolytic enzymes a practical approach., Beynon and Bond Eds 5 Oxford University Press, England, 87-88).

[0079] The results above show the low affinity of the same by NEP, suggesting a possible decrease of the collateral effects such as angioedema and cough when compared to the vasopeptidases inhibitors (omapatrilat for example) related in 10 the state of the technique.

Example 3: EVASINS BRADYKININ POTENTIATING ACTIVITY TEST - BIOLOGICAL ASSAY IN GUINEA PIG ISOLATED ILEUM

[0080] The potentiating activity of synthetic peptides using 15 the smooth muscles contractile activity induced by bradykinin was tested and the UP values were determined using the guinea pig isolated ileum preparation. The UP corresponds to the Evasin concentration (nmol/mL of preparation) that is able to change the response effect of a single bradykinin dose into an 20 equivalent double dose.

[0081] Female guinea pigs were used. Before starting the assays, the ileum was kept in a Tyrode solution. Next, one of the ends of the ileum segment, measuring 1.5 to 2.0 cm, was tied up to a semi-ring contained in the bottom of a glass cup 25 containing Tyrode saline at 37°C with constant oxygen bubbling using a capillary; the other end was secured to a previously calibrated lever. The tension maintained was 1 g and the guinea pig isolated ileum contractions were recorded. So as to determine the samples potentiating effect on bradykinin, a 30 log-dose response curve of the bradykinin effect on the guinea pig isolated ileum was plotted. The bradykinin activity is determined by measuring the guinea pig isolated ileum

contractions and the potentiating activity is expressed in terms of an increased tissue response to a standard bradykinin dose as per Shimuta et al., Eur. J. pharmacol. 70 (4), 551-554 (1981).

5 | [0082] The Evasins were individually tested and spiked before the addition of a single bradykinin dose. The samples dilutions were prepared using deionized water upon use. The measured response was interpolated on the linear portion of the log dose-effect curve, thus obtaining the potentiating 10 activity in terms of an increase in the preparation response for a standard bradykinin dose. TYRODE solution: stock solution I 20 mL, stock solution II 40 mL, diphenhydramine solution (1 mg/mL) 1 mL, atropine solution (1 mg/mL) 1 mL, 5.60 mM D-glicose and H₂O q.s. 1 L. All reagents used in this 15 assay were analytical-grade reagents.

| [0083] All Evasins potentiated the contractile action of bradykinin in guinea pig isolated ileum, duplicating the contractile effect of bradykinin at concentrations ranging from 0.22 to 30 nmols.

20 **Example 4: ACTION OF EVASINS ON THE BLOOD PRESSURE OF ANESTHETIZED RAT**

| [0084] The potentiating activity of the bradykinin hypotensive effect was tested in anesthetized rats. Normotensive male rats (WKY) were anesthetized using pentobarbital sodium (Hypnol® 25 Cristália, 50 mg/kg, intraperitoneal) and placed on a controlled-temperature board for maintaining the body temperature between 36.5°C and 37°C. A polygraph coupled to a physiological transducer was used. The blood pressure ranging values were obtained by integrating the areas limited by the 30 baseline pressure and comparing them to the values obtained from control assays. In in vivo assays, the potentiating activity of Evasins on the hypotensive effect of bradykinin on anesthetized rat blood pressure was observed. Two parameters

were determined for comparison of the potentiating effects of bradykinin on the anesthetized rat blood pressure ($n = 5$):

1) Intensity of the potentiating effect of the hypotensive activity of bradykinin on the anesthetized rats blood pressure: This value was defined as the hypotension percentage (%) increase caused by a single dose of bradykinin obtained after the infusion of 200 nmol of potentiator. 2) Potentiating effect length: Time required to reduce the potentiating effect on a single dose of bradykinin by 50%.

10 | [0085] The hypotensive effect of bradykinin was potentiated by Evasins in the range from 40 to 340% in anesthetized rats by intravenous injection, at a steady concentration of 200 nmoles of Evasin/rat. A minimum length of 10 minutes was observed and even exceeded 120 minutes for a reduction of the initial 15 | potentiating effect by 50%.

| Please replace Paragraph [0086], with the following rewritten paragraph: [0086]

Table 3: Potentiation by the Evasins of the bradykinin hypontensive effect on anesthesiated rats blood pressure

PEPTIDE	Bk potentiation (%)	Duration time
EVASIN-5a, SEQ ID No. <u>10</u>	102,5	10
EVASIN-7a, SEQ ID No. <u>253</u>	41,8	120
EVASIN-10c, SEQ ID No. <u>472</u>	195	95
EVASIN-11b, SEQ ID No. <u>552</u>	164,3	90
EVASIN-11c, SEQ ID No. <u>553</u>	67	28
EVASIN-11e, SEQ ID No.	41	35

<u>555</u>			
EVASIN-12b, <u>SEQ ID No.</u>	340	40	
<u>557</u>			
EVASIN-13a, <u>SEQ ID No.</u>	45	30	
<u>590</u>			

**Example 5: PREPARATION OF THE INCLUSION COMPOUND BETWEEN
 β -CYCLODEXTRINS AND THEIR DERIVATIVES AND THE EVASINS AND
THEIR ANALOGUES**

5 | Please replace Paragraph [0087], with the following rewritten
paragraph:

This example presents the characterization of the inclusion compound between HP- β cyclodextrin and the evasin 5a, SEQ ID No. 10, such as no limitant example. The preparation is made in molar ratios of β -cyclodextrin and their derivatives and 10 Evasins and their analogs in aqueous solutions (1:1 and 1:2). The solutions mixture is subject to constant stirring up to the complete dissolution of β -cyclodextrin. Subsequently, the mixture is frozen at liquid nitrogen temperature and subject to the lyophilization process for 24 hours. The solid thus 15 obtained was characterized using physicochemical analysis techniques. Nuclear magnetic ressonance was the technique providing relevant information on the interaction host/guest.

[0088] The preparation was made in equimolar ratios of cyclodextrin and peptides. The table below shows the inclusion 20 compounds so prepared. These systems were subjected to biological tests.

Please replace Paragraph [0089], with the following rewritten paragraph:

**Table 4: Inclusion compounds between cyclodextrins and Evasins
that were submitted to biological tests.**

EVASIN	Cyclodextrin
EVASIN-5a, <u>SEQ ID No.</u>	β -Cyclodextrin

<u>10</u>	
<u>EVASIN-7a, SEQ ID No.</u>	β -Cyclodextrin
<u>253</u>	
<u>EVASIN-9a, SEQ ID No.</u>	β -Cyclodextrin
<u>326</u>	
<u>EVASIN-10c, SEQ ID</u>	β -Cyclodextrin
<u>No. 472</u>	
<u>EVASIN-5a, SEQ ID No.</u>	HP- β -CD
<u>10^a</u>	
<u>EVASIN-7a, SEQ ID No.</u>	HP- β -CD
<u>253^a</u>	
<u>EVASIN-9a, SEQ ID No.</u>	HP- β -CD
<u>326^a</u>	
<u>EVASIN-10c, SEQ ID</u>	HP- β -CD
<u>No. 472</u>	
<u>EVASIN-12b, SEQ ID</u>	HP- β -CD
<u>No. 557</u>	

Please replace Paragraph [0090], with the following rewritten paragraph:

After the preparation of solutions and the simulation of the NMR ^1H and ^{13}C spectra of HB- β -CD and peptides, the NMR-characterization step of the inclusion compound HB- β -CD/BPP-5a, as well as free BPP-5a and HB- β -CD free. Hydrogen - ^1H NMR, to structural elucidation of the cyclodextrins and host molecules BPP-5a, SEQ ID No. 10, + COSY, TOCSY, ^{13}C -DEPT135, HMQC to structural elucidation of BPP-5a structural; variations analyses of chemical dislocation - δ ; time measurements of the longitudinal relaxation - RMN T₁ and NOESY experiments (Nuclear Overhauser Effect Spectroscopy), heading to verify the inclusion. δ and T₁ study utilized as a probe only the hydrogens of the host molecules as the cyclodextrin hydrogen signal are overlapped.

Please replace Paragraph [0091], with the following rewritten paragraph:

To structural elucidation of the pentapeptide BPP-5a, SEQ ID No. 10, to RMN, was utilized as reference, the RMN ^1H spectral of the specifics free aminoacids (THE SADTLER STANDARD SPECTRA. Sadtler Research Laboratories. 1972). Due to a complexity of the molecule, as well as its spectral it was necessary the use of many RNM techniques as one-dimensional as bi-dimensional, being also necessary to use 400 MHz spectrometer. The sample was dissolved in D_2O , the spectral based in the hydrogen nuclear magnetic resonance were very simplified, in function of the hydroxy, amida and amina groups hydrogen signals absence, once that they are changed to the deuterium atoms of the solvent.

Please replace Paragraph [0092], with the following rewritten paragraph:

Based on the corresponding simulations for Evasin-5a, the actual spectrum of the pure compound was analyzed, the following being assigned:

- 20 1) The NMR ^1H spectrum, as well as the COSY, showed an intense signal at 4.8 ppm, as a result of the presence of the impurity " H_2O " in the deuterated solvent; -
- 25 2) The "CH" groups hydrogens are next to the water signal; these hydrogens are a link between the peptide bonds and the amino acids functional groups; -
- 30 3) The "CH₂" groups are present within the region comprised between the chemical displacement at 2.8 - 3.7 ppm, these groups being bonded to the "CH" groups next to the peptide bonds; -
- 4) Between 1.3 - 2.5 ppm approximately, multiplets referring to "CH₂" that belong to several functional groups can be seen; -

5) At 1.2 ppm, a doublet referring to the CH₃ group of the segment of the amino acid Alanine can be seen. Using COSY, the scalar coupling of this group with a "CH" at 4.6 ppm (quartet) can be observed; -

5 6) The region comprised between the chemical displacement range from 7.7 to 7.1 ppm was assigned to the aromatic group spins system (corresponding to the segment of the amino acid Tryptophan); -

10 [0093] The HP- β -CD spectrum showed to be highly complex, difficult to assign, even when based on the simulated spectrum. However, it is possible to assign some signals mentioned below:

15 [0094] Between the chemical displacements at 3.3 to 4.3 ppm, a complex multiplet is seen as a result of the "CH" groups of carbons bonded to hydroxyls. At the chemical displacement interval between 1.1 to 1.8 ppm, the signals referring to the hydroxypropyl groups can be found.

Please replace Paragraph [0095], with the following rewritten paragraph:

20 Concerning the NMR assay of the inclusion compound, comparing the pure Evasin-5a spectrum, observed a clear separation of spectral lines, with a consequent chemical displacement variation within the aromatic groups region ($\delta \approx 7.7 - 7.1$), .

25 This is a strong indication of the interaction of the HP- β -CD with this region of this peptide. This phenomenon is due to the electrons no ligants of the atoms of oxygen C1-O1-C4 of the cyclodextrin that can cause a disturbance in the electronic distribution of the aromatic groups of the peptide. This result suggests the possible encapsulation of the 30 aromatic group into the HP- β -CD cavity.

[0096] In relation to the Evasin-5a, presented in the table 1 and 2 (values of T1), observed that only the hydrogen 16 and

19 had significant variations, once that the T1 variation of this nucleus is much bigger than the standard deviation obtained in each measurement. The others hydrogens analyses presented T1 variations that are questionable if compared to 5 the standard deviation of each measurement.

[0097] Analyzing the tables below, it was observed a reduction of the relaxation time, T1, of the host molecules aromatics hydrogens. This alteration suggests a reduction of the mobility of the guest molecules, a symmetry change and a slow 10 rotational kinetic after the inclusion of the host molecules in the cavity of the cyclodextrin.

[Please replace Paragraph [0098], with the following rewritten paragraph:

Table 5: Values of longitudinal relaxation time of some nuclei 15 de 1 H of the peptide BPP-5a pure, SEQ ID No. 10, measure in 200 MHz.

Group number	Chemical displacement	Relaxation time (T1)
19	7,5052 ppm	0,7603 ± 0,0078 s
16	7,4068 ppm	1,2927 ± 0,0122 s
21	4,3592 ppm	0,4147 ± 0,0343 s
14	3,1657 ppm	0,1778 ± 0,0031 s
24	2,8750 ppm	0,5969 ± 0,0025 s
9 e 10	1,8857 ppm	0,2864 ± 0,0090 s

[0099] **Table 6:** Values of the longitudinal relaxation time of some nuclei of the 1H of the system HP- β -CD/BPP-5a, measured 20 in 200 MHz

Group number	Chemical displacement	T1 / s	ΔT_1 / s
19	7,5353 ppm	0,5342 ± 0,0046	-0,2261
16	7,4063 ppm	0,7127 ± 0,0043	-0,5800
21	4,3760 ppm	0,3533 ± 0,0670	-0,0614
14	3,2020 ppm	0,1518 ± 0,0129	-0,0260
24	2,8890 ppm	0,5182 ± 0,0011	-0,0787
9 e 10	1,6040 ppm	0,2634 ± 0,0046	-0,0230

Please replace Paragraph [0100], with the following rewritten paragraph:

It was possible to determine the guest molecules spacial arrangement inside of the cyclodextrins cavity using the NOESY 5 of β -CD e BPP-5a/HP- β -CD experiments, adding to the others RNM data. The NOESY experiment was carried out with D8 = 650 ms to the BPP-5a, SEQ ID No. 10. This was the unique value of D8 that observed cross correlations that could indicate a spacial proximity between the HP- β -CD and BPP-5a hydrogens 10 as example no limitant.

Example 6: EFFECT OF EVASINS ON THE BLOOD PRESSURE OF NON ANESTHETIZED HYPERTENSIVE RAT

[0101] One day before the assay, the animals were subjected to a surgery for cannulation of the femoral artery and vein. The 15 rats were anesthetized with ether and placed in dorsal decubitus on a surgical board. A small skin incision was made, thus separating the musculature for locating the femoral vasculonervous bunch. The cannulas were inserted into the inferior vena cava through the femoral vein for administering 20 the drug and into the abdominal aorta through the femoral artery for recording the cardiovascular parameters. After insertion, the cannulas were tied to the bunch using a surgical line. Next, the cannulas were directed subcutaneously with the aid of a trocater to the scapular waist where they 25 were exteriorized and secured using a suture line. The arterial cannula was used for recording the cardiovascular parameters and the venous cannula for administering the drugs.

[0102] The blood pressure and the heart rate were recorded one day after the femoral artery and vein cannulation. The assay 30 was conducted with non-anesthetized animals with free movement. The rats pulsed arterial pressure (PAP), the mean

arterial pressure (MAP) and heart rate (HR) were monitored by a computer, using a data acquisition system (BIOPAC). The data were collected during all the experiments.

5 | Please replace Paragraph [0103], with the following rewritten paragraph:

Before the drug administration, the rats PBP, MBP and HR were monitored. After the bolus injection of Evasin in a total volume of 0.2 mL (NaCl 0.9% solution), the resulting hypotensive effect and the effect length were monitored. In 10 this assay, standard doses of each peptide (70 nmol /100 g of body weight) were tested (n = 4). Evasin-10c, SEQ ID No. 472, was the most potent peptide with the best hypotensive effect, producing a reduction of -29.5 ± 9.50 mmHg in the mean blood pressure, followed by Evasin-9a, SEQ ID No. 326, with a MBP 15 reduction of -27.0 ± 1.50 mmHg. Evasin-5a, SEQ ID No. 10, -and the Evasin-7a, SEQ ID No. 253, produced a maximum MBP reduction of -19.17 ± 4.36 mmHg and 13.00 ± 2.78 mmHg respectively.

20 | Please replace Paragraph [0104], with the following rewritten paragraph:

When comparing the effect length, it was observed that Evasin-7a, SEQ ID No. 253 was the peptide showing a hypotensive effect lasting more than 100 minutes. Evasin-9a, SEQ ID No. 326, and Evasin-10c, SEQ ID No. 472, were similar in terms of 25 effect length of 84 and 94 minutes, respectively. The effect length of Evasin-5a, SEQ ID No. 10, was short; 37 minutes after the administration the MAP returned to the baseline values.

**Example 7: EFFECT OF ENCAPSULATED EVASINS IN
30 CYCLODEXTRINS ON THE BLOOD PRESSURE OF AWAKE HYPERTENSIVE RAT**

| [0105] This example describes the inclusion compound administration effect between the Evasin-5a and hidroxiprolil-

β-cyclodextrin (HPβCD) on the blood pressure of the hypertensive rats. One day before the assay, the animals were subjected to a surgery for cannulation of the femoral artery and vein. The rats were anesthetized with ether and placed in 5 dorsal decubitus on a surgical board. A small skin incision was made, thus separating the musculature for locating the femoral vasculonervous bunch. The cannulas were inserted into the inferior vena cava through the femoral vein for administering the drug and into the abdominal aorta through 10 the femoral artery for recording the cardiovascular parameters. After insertion, the cannulas were tied to the bunch using a surgical line. Next, the cannulas were directed subcutaneously with the aid of a trocater to the scapular waist where they were exteriorized and secured using a suture 15 line.

[106] The blood pressure and the heart rate were recorded one day after the femoral artery and vein cannulation. The assay was conducted with non-anesthetized animals with free movement. The animals pulsed arterial pressure (PAP), the mean 20 arterial pressure (MAP) and heart rate (HR) were monitored by a computer, using a data acquisition system (BIOPAC). The data were collected during all the experiments.

[0107] Before the drug administration, the rats PBP, MBP and HR were monitored during 60 minutes. After the bolus injection 25 of Evasin in a total volume of 0.2 mL (NaCl 0.9% solution), the resulting hypotensive effect and the effect length were monitored. A standard doses of 0,071 nmol /100 g of body weight, n = 6, was utilized in this assay.

30 | Please replace Paragraph [0108], with the following rewritten paragraph:

| When comparing the results obtained from the administration of
| free Evasin-5a, SEQ ID No. 10, and the Evasin-5a included in
| HPβCD, no differences were observed in the maximum hypotensive

effect between the administration of the free pentapeptide or in the encapsulated form, reduction of MAP 23 ± 4.2 mmHg and 22 ± 3.3 mmHg, respectively. We observed a relevant difference in the effect length, where the encapsulated Evasin-5a was 5 able to increase the free peptide effect length by more than 4 times than the duration time of the free peptide, 140 and 38 minutes respectively.

Example 8: COMPARATIVE EFFECT OF THE EVASINS ON THE HYPERTENSIVE RATS AWAKED BLOOD PRESSURE (SHR AND TGR(mREN2)L27

10 | [0109] This example, no limitant, describes the infusion effect of the Evasin-5a on hypertensive rats blood pressure of different strain.

15 | [0110] One day before the assay, the animals were subjected to a surgery for cannulation of the femoral artery and vein. The rats were anesthetized with ether and placed in dorsal decubitus on a surgical board. A small skin incision was made, thus separating the musculature for locating the femoral vasculonervous bunch. The cannulas were inserted into the inferior vena cava through the femoral vein for administering 20 the drug and into the abdominal aorta through the femoral artery for recording the cardiovascular parameters. After insertion, the cannulas were tied to the bunch using a surgical line. Next, the cannulas were directed subcutaneously with the aid of a trocater to the scapular waist where they 25 were exteriorized and secured using a suture line.

30 | [0111] The blood pressure and the heart rate were recorded one day after the femoral artery and vein cannulation. The assay was conducted with non-anesthetized animals with free movement. The animals pulsed arterial pressure (PAP), the mean arterial pressure (MAP) and heart rate (HR) were monitored by a computer, using a data acquisition system (BIOPAC). The data were collected during all the experiments.

Please replace Paragraph [0122], with the following rewritten paragraph:

Before the drug administration, the rats PBP, MBP and HR were monitored during 60 minutes. During and after the infusion of 5 the Evasin 5a, SEQ ID No. 10, 900 µg/100g corporal weight /hour in NaCl solution 0.9% (n = 6), the cardiovascular parameters were registered. The administration of the evasin-5a in both strains produced the same decreased of the MAP 28 ± 2.7 mmHg and 34 ± 0.9 mmHg in the SHR and TGR (mREN2) L27 10 respectively. However, it was observed that the relevant difference in time duration effect after the infusion interruption was over 6 hours in the SHR and only 1 hour in the TGRs. These differences indicate important variations of 15 the biochemical mechanisms of the Evasins 5a action with different degree for the renin angiotensin system activation.

Example 9: EFFECT OF ACUTE ADMINISTRATION OF THE EVASINS ON SHR BLOOD PRESSURE MEASURED BY TELEMETRY SYSTEM

[0113] A telemetry system was used for measuring the systolic and diastolic pressure, the mean blood pressure and the heart 20 rate. This monitoring system consists in an implantable radio frequency device, a receptor board, a matrix and a computer with a software for data acquisition and analysis (Braga, et al., 2002).

[0114] Male SHR rats were used. The animals remained fasted 25 for 24 hours before the surgery. Under anesthesia using 2.5% 2,2,2-tribromoethanol (1 mL/100 g of body weight), the rats were placed in dorsal decubitus on a surgical board, the ventral abdominal region was clipped and aseptically cleaned with iodated alcohol. An incision of about 2 cm in the median 30 abdominal line was made so as to have a good view of the iliac bifurcation area. The bowels were removed so as to allow a complete access to the abdominal blood vessels. The adipose

and connective tissues along the vascular bed were delicately removed with the aid of cotton swabs and gauzes until the abdominal aorta could be identified and properly isolated from the vena cava. A cordonet thread wetted with saline embraced 5 the aorta so as to prevent the blood flow and a small cut was made using the needle (25 x 8) bent in an angle of 90°. Next, using a guide pincer, the device polyethylene catheter received the biocompatible gel and was inserted into the artery. The catheter inlet area was cleaned and dried and a 10 tiny amount of tissue glue was applied. Over the glue, a small piece of cellulose paper was placed to secure the catheter in the aorta. The device battery was started with the magnet and, using an AM radio (not tuned), the typical sound of the catheter position in the aorta was recorded. The abdominal 15 musculature was sutured, securing the device by its silicone tab. Next, the skin was sutured. Asepsis was made using iodated alcohol and 0.1 mL of pentabiotic was then administered intramuscularly. The animals were placed in individual cages and remained under warmed conditions until 20 totally recovered from anesthesia. After recovery, the animals were taken to the telemetry room climatized at 25°C and maintained on a 12 hr/12 hr light/dark cycle (6:00 a.m. to 6:00 p.m. day and 6:00 p.m. 6:00 a.m. night). Water and food were given *ad libitum*.

25 | [0115] Before proceeding to the assay, the animals were placed in individual cages (15 cm x 12 cm x 6 cm) and kept for 8 days until the telemetry plots indicated blood pressure and heart rate recovery. The data was sampled every 10 minutes for 10 seconds/24 hours.

30 | Please replace Paragraph [0116], with the following rewritten paragraph:

The results obtained, by oral administration, of the Evasin-7a, SEQ ID No. 253, and Evasin-10c, SEQ ID No. 472,

encapsulated in β -cyclodextrin gavage, showed that these two peptides exert a hypotensive activity in spontaneously hypertensive rats. Evasin-7a, SEQ ID No. 253, showed a length of 9 hours, with a maximum reduction of the MAP of 20 mmHg after 5 hours and within 8 mmHg after the peptide administration and 5 hours, respectively. Evasin-10c, SEQ ID No. 472, had a maximum hypotensive effect of 5 hours and a maximum reduction of 13 mmHg, two hours after administration.

Example 10: BIODISTRIBUTION OF EVASINS

[0117] The intravenous administration of ^{125}I labeled Evasins according to the chloramine T method in mice showed that these peptides are noticeably concentrated in the kidneys. For example no limitant, the intravenous injection of ^{125}I - Evasin10c showed, after 15 minutes, that this peptide had a concentration about twice as high (per gram of tissue) in the kidneys than in the lungs and liver. In other tissues and blood, the radioactive peptide concentration was significantly lower. This difference increases in the kidneys when compared to other tissues, peak concentrations are reached after 30 minutes, drop quickly in tissues and much slower in the kidneys where they remain at about 50% of the peak concentration observed 3 hours after the administration. This same distribution profile was seen when the radioactive peptide was administered together with captopril at a molar concentration 10 times higher than the peptide concentration, reducing about 30% of the peak concentration reached by the radioactive peptide when compared to that reached without captopril.

[0118] Both the biodistribution and the dwelling time of Evasins in the kidneys showed that they have a greater selectivity for the kidneys and remain bonded to that tissue for a longer period of time than captopril. To the other hand,

the 30% reduction of the renal binding of the Evasins is ligands to the angiotensin-converting enzyme.